

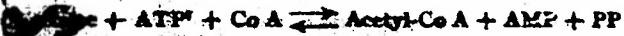
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PARTICIPATION OF THIOCTIC ACID IN THE ACETATE-ACTIVATING REACTION¹

The well-known role of thioctic acid as an acyl carrier in the oxidation of α -keto acids^{2,3} suggests that this cofactor may participate in other acyl transfer reactions. Accordingly, extracts of pigeon liver powder were examined for a thioctic acid requirement in the acetate-activating reaction:



Extracts were prepared by grinding 1 g. of powder with 10 ml. of 0.2 M NaHCO₃ and the insoluble material was removed by centrifuging at 2,000 \times g for 30 min. Co A was removed from the supernatant by treatment with Dowex-1.⁴ The crude extracts respond to the alumina procedure⁵ for the removal of significant quantities of bound thioctic acid from the enzymes. Although assay⁶ shows that only about 50% of the thioctic acid is removed by the alumina procedure, treated enzymes possess significantly less acetate activating activity, as measured by the hydrazinic acid method,¹⁰ than do untreated enzymes (Table I). This indicates that although the cofactor has not been completely removed, the concentration has been decreased to below enzyme saturation level. Activity of the acetate-activating system may be restored to alumina treated preparations by the addition of 7.5 μ g. of synthetic thioctic acid.¹¹

Two fractions of the pigeon liver extracts have been separated by ammonium sulfate precipitation; the fractions were collected by centrifugation at 18,000 \times g for 15 min. Fraction I (0-35% saturated) contains only very slight acetate activating activity. Fraction II (35-70% saturation) contains almost the entire activity contained in the crude extract; but it does not respond to alumina treatment. Removal of thioctic acid by the alu-

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(7) The following abbreviations are used: ATP, adenosine triphosphate; Co A, coenzyme A; AMP, adenosine monophosphate; PP_i, pyrophosphate; TRIS, tri-(hydroxymethyl)-aminomethane.

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(11) Kindly supplied by Dr. R. L. R. Stadtman.

TABLE I

Incubation mixture contained, per 1.4 ml.: 100 μ M. Na acetate; 20 units Co A; 200 μ M. TRIS buffer, pH 8.2; 10 μ M. glutathione; 200 μ M. hydroxylamine; 10 μ M. Na-ATP; 10 μ M. MgCl₂; 60 μ M. NaF; enzyme solution. Incubated 30 min. at 35°. Glutathione, Na-ATP and hydroxylamine were adjusted to pH 8 with 1 M TRIS before use. Thioctic acid content of crude untreated material was 20 μ g. per mg. protein; after alumina treatment the content was 11 μ g. per mg. Fraction I and II contained 17 μ g. thioctic acid per mg. protein before alumina treatment and 9 μ g. per mg. after treatment. Alumina treatment of Fraction II alone resulted in no change in thioctic acid content.

Additions and treatment	M. thioctic acid added			
	None	Crude (0.3 μ g. thioct. II)	Crude (0.3 μ g. thioct. II + 7.5 μ g. thioct. II)	Alumina treated (0.3 μ g. thioct. II)
7.5 μ g. thioctic acid	0.96	0.02	0.53	0.54
Alumina treated	.4953	.54
Alumina treated + 7.5 μ g. thioctic acid	.8047
2 μ M. arsenite	.8848	.49
Alumina treated + 7.5 μ g. thioctic acid + 2 μ M. arsenite50
Alumina treated + 7.5 μ g. thioctic acid + 2 μ M. arsenite + 20 μ M. cysteine	4640
Alumina treated + 7.5 μ g. thioctic acid + 2 μ M. arsenite + 20 μ M. added glutathione	.5039
Alumina treated + 7.5 μ g. thioctic acid + 2 μ M. arsenite + 10 μ M. BAL	.8450

mina procedure is achieved upon combination of the two fractions. Heating Fraction I for 10 min. at 65° completely destroys the activity. It thus appears that Fraction I functions by splitting protein-bound thioctic acid from the enzyme; liberated thioctic acid is then adsorbed and removed by the alumina.

As anticipated¹² with a reaction requiring thioctic acid, the acetate-activating system is inhibited by arsenite, but not by arsenate. The arsenite sensitivity of systems containing alumina-treated enzyme plus added thioctic acid is much greater than is the sensitivity in mixtures with untreated enzymes containing an excess of the cofactor. The inhibition is reversed by BAL, but not by such monothiols as cysteine or glutathione.

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